IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	**Rocklage et al.**	Examiner:	**Hartley**
Serial No.:	**09/189,043**	Group Art Unit:	**1619**
Filing Date:		Docket No.:	**1897**
Title	**Method of Perfusion Imaging**		

Date of Deposit: 4/3/02
I hereby certify that this paper is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231

Signature: _____
Printed Name: Robert C. Beck

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Rule 131 Declaration

Assistant Commissioner for Patents
Washington, DC 20231

We, John Kucharczyk and Michael Moseley, declare:

1. We make the following declaration of our own personal knowledge, and if required, could competently testify to the matters contained herein.

2. Prior to October 1989, we completed the following experiments, which reduced to practice the invention claimed in the pending patent application. We completed the following experiments at the University of California, in San Francisco, California, in the United States. Attached hereto is a true copy of our laboratory notebook, with the dates deleted. All of the deleted dates are prior to October 1989.

3. We were the inventors of the inventions described below and in the attached laboratory notebook exhibits. Dr. Rocklage is named as a co-inventor on the pending patent application. However, Dr. Rocklage was not involved and did not contribute to the inventions described below and in the attached laboratory notebook exhibits.

4. We conducted experiments on laboratory cats approved by the UCSF Committee on Animal Research. These experiments were designed to determine whether we could detect blood flow abnormalities using magnetic resonance ("MR") imaging. In the first experiment shown in the laboratory notebooks, we did a surgical occlusion of the middle cerebral artery on one side of the brain of a cat at 1:13 p.m. This occlusion created an obstruction in the blood vessel and induced a blood flow abnormality in the middle cerebral artery and the region of the brain which receives blood flow from the middle cerebral artery. We then obtained several series of temporally-spaced MR images from the brain of the cat that demonstrated a blood flow abnormality in the brain tissue

5. The laboratory notebooks show that the first series of T2-weighted MR images was obtained at 1:40 p.m., prior to administering the contrast agent. At 1:45 p.m. we administered MR contrast agent by injecting Dy-DPTA into a laboratory cat. (We called this contrast agent S–043 because we had obtained the contrast agent from Salutar, and Salutar used the code-name "S-043" to designate Dy-DPTA). The contrast agent was injected into the vasculature of the cat by intravenous injection into the cephalic vein of the anesthetized animal. We obtained a series of temporally spaced T2-weighted MR images of the cat brain. The first series of images was obtained from 1:45 p.m. to 1:57 p.m. The second series of images was obtained from 1:59 p.m. to 2:11 p.m. The third series of images was obtained from 2:12 p.m. to 2:23 p.m. The fourth series of images was obtained from 2:47 p.m. to 2:60 p.m. The notebook

records indicate that the contrast agent was injected before, during, and after the MR image acquisition.

6. A second injection of contrast agent was administered, and we obtained another series of temporally spaced images beginning at 4:35 p.m.

7. These temporally spaced series of MR images showed variations in signal intensity in the brain. These variations were observable because the contrast agent allowed us to visualize the blood flow abnormality on the side of the brain affected by occlusion of the cerebral artery. On the other side of the brain, the cerebral artery was not occluded. Blood flow on the non-occluded side of the brain remained normal and could be compared with the blood flow abnormality on the side with the occluded middle cerebral artery.

8. At 7:05 p.m. the cat was euthanized, and the brain was removed and put it in TTC (tetrazolium triphenyl chloride). TTC is a well-recognized enzymatic staining method for accurately quantifying the location and extent of damage to the brain after the animal is euthanized. We used TTC staining to corroborate that the signal intensity and spatial location information we obtained from the MR images was accurately portraying the location and extent of the blood flow abnormality caused by the induced stroke.

8. Our laboratory notebook for that day contains the statement

"Coronal sections 5-7 mm thick show infarcted tissue through MCA-O ® territory

Close correspondence with T2W MRI app[?]"

9. These statements show that we immediately recognized that following intravenous injection of Dy-DPTA the signal attenuation induced by Dy-DPTA varied regionally within the brain as a function of the blood flow to that region. Brain regions with higher blood flow, such as the gray matter of the cerebral cortex, had a reduced signal intensity compared to

adjacent white matter. By comparison with normal brain tissues, we observed that the wash-in, wash-out transit characteristics of the contrast agent in ischemic brain regions was variably delayed, such that the degree of signal attenuation caused by the contrast agent directly reflected the severity and spatial extent of the brain region affected by the induced stroke.

10. Our laboratory notebook on that date states "DO ROIs" and references "washout curves." ROI stands for region-of-interest. "DO ROIs" meant that we intended to later confirm though computer region-of-interest calculations the signal intensity changes we observed on the MR images. We also used a calibrated grid measurement planimeter which we had developed. The grid is placed over the brain image on the computer screen in order to measure the area of stroke injury in the sequential temporally-spaced images.

11. Attached as Exhibit B is a true copy of our laboratory notebooks showing that we repeated the above experiment several more times. Each time we observed the same phenomena. That is, that we could obtain a series of temporally spaced images that showed an entire brain slice. By observing the signal change in a given area of the brain in the temporally spaced images we could determine the location and extent of blood flow abnormality.

We declare under penalty of perjury under the laws of the Unites States that the foregoing is true and correct.

Executed on _____, 2002 in _____, Georgia

By: _____
Dr. John Kucharczyk

Executed on _____, 2002 in _____, California

By: _____
Dr. Michael Moseley